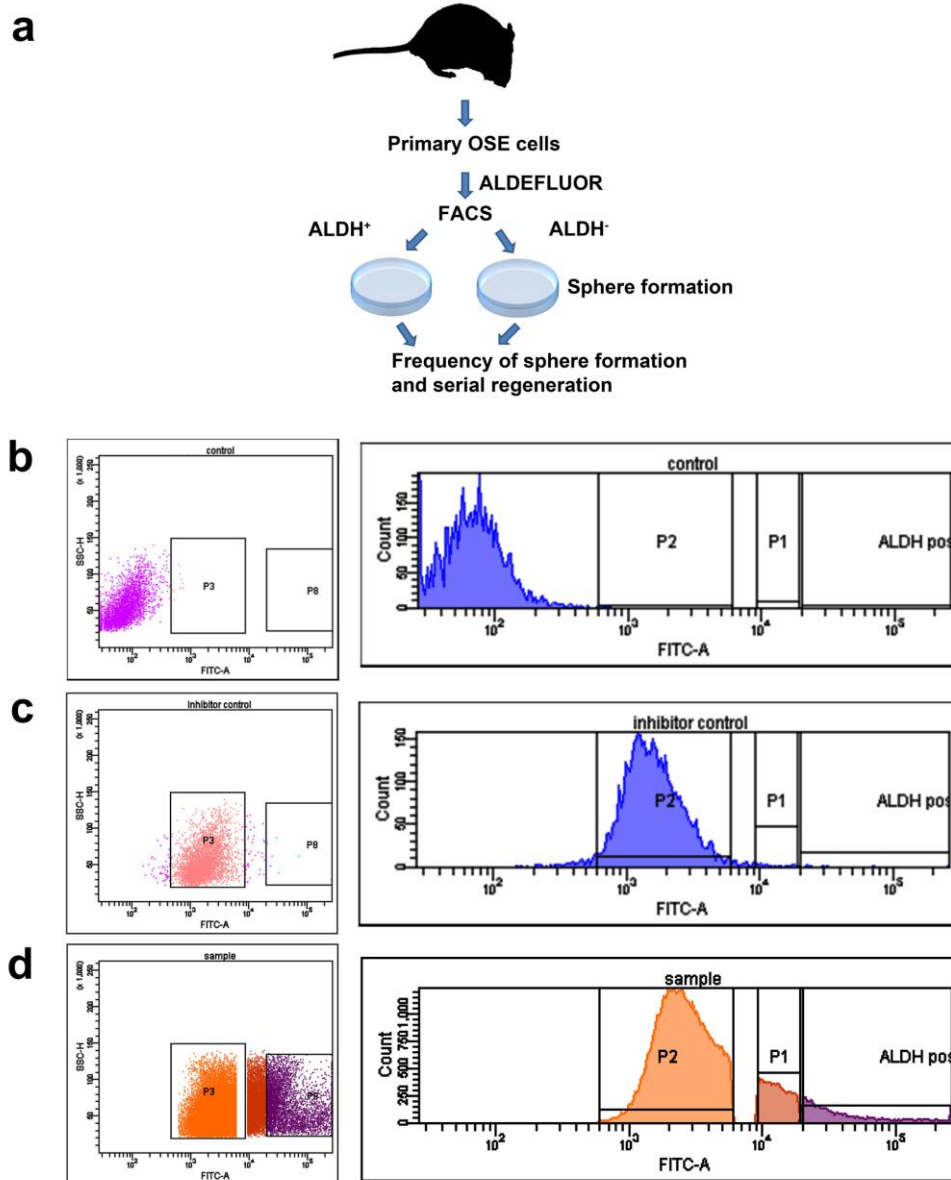
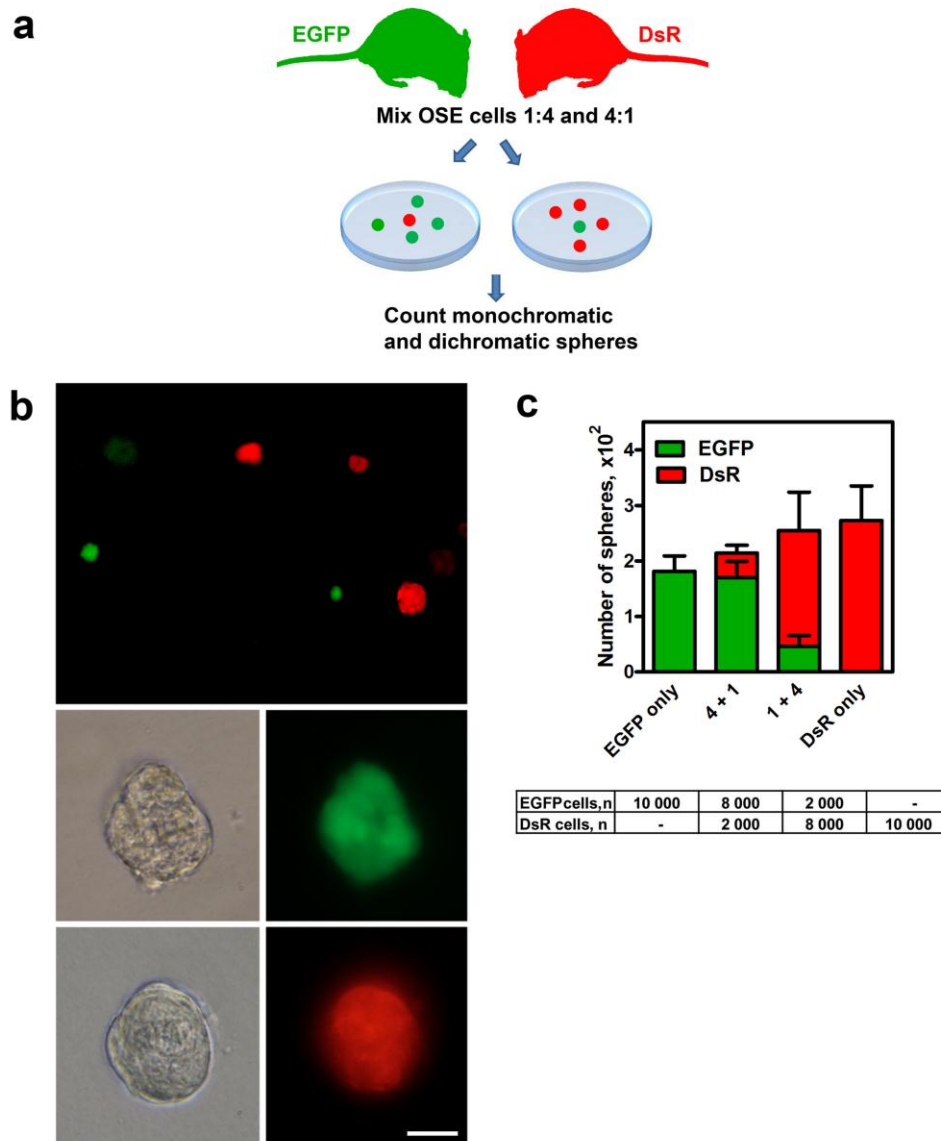


Supplementary Figures and Legends

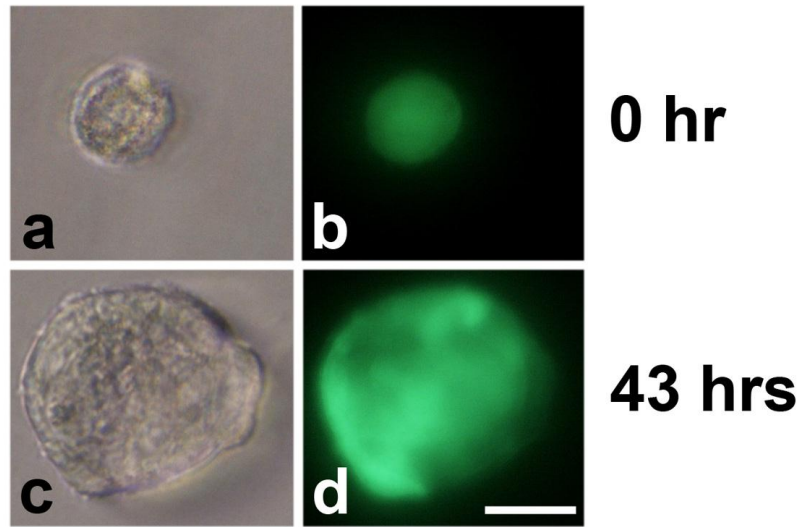


Supplementary Figure 1. Isolation of ALDH⁺ and ALDH⁻ OSE cells in FACS experiments.

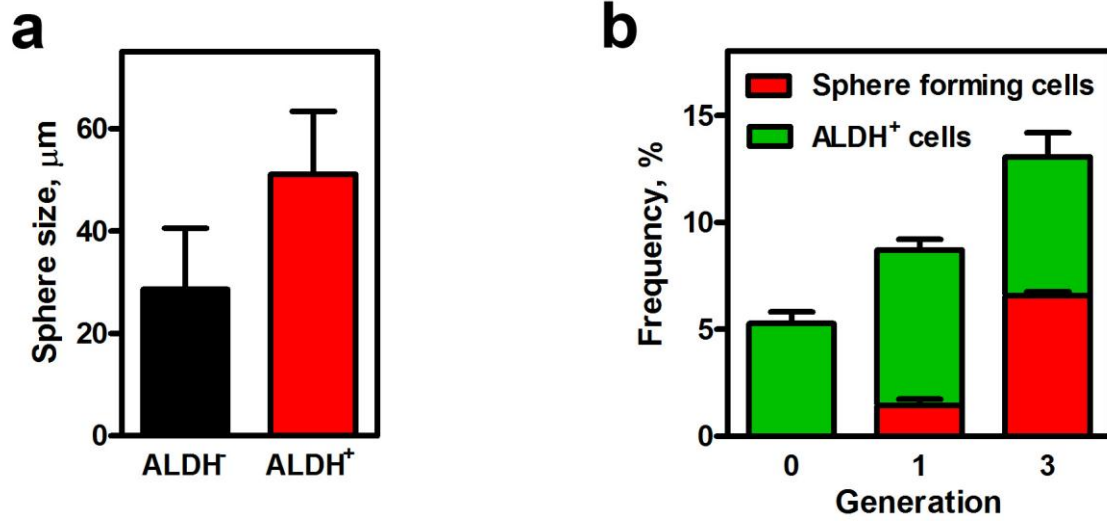
a, Experimental design. Primary OSE cells were treated with ALDEFLUOR, separated according to level of ALDH enzymatic activity by FACS and assessed for frequency of sphere formation and serial regeneration (dissociation/clonal formation). **b-d**, Gating profiles of ALDH⁺ (ALDH pos) and ALDH⁻ cells. Dot plot of side-scatter (SSC-H) and light-scatter set on FITC-A channel 488 nm (left) with the corresponding histogram (right) of untreated (b), treated with ALDEFLUOR and ALDH inhibitor (c) or treated with ALDEFLUOR OSE cells (d). ALDH⁻ cells are gated as P3 (dot plot) and P2 (histogram), ALDH⁺ are gated as P8 (dot plot) and ALDH pos (histogram). In all samples PI was added for exclusion of dead cells.



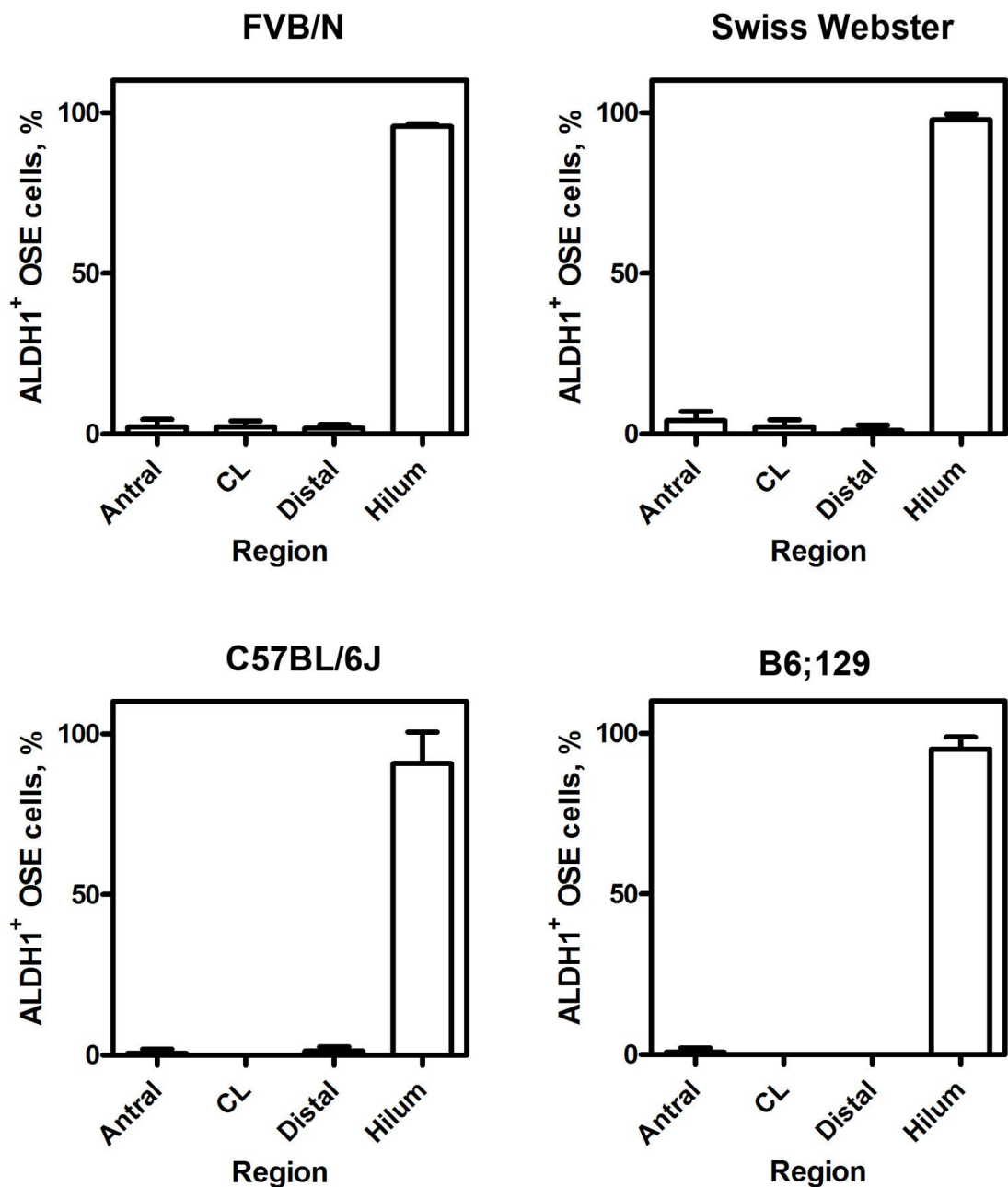
Supplementary Figure 2. Monoclonality of OSE spheres. **a**, Experimental design. Single cell suspensions of primary OSE were derived from of Tg β -actin EGFP or β -actin DsRed mice, mixed at various ratios, placed in Geltrex/OSE-SCM around the rim of a well of a 12 well plate and grown for 7 to 12 days. **b**, Fluorescence images of DsRed (red) and EGFP (green) positive monoclonal OSE-spheres (top). Phase contrast (left) and fluorescence (right) of individual spheres (middle and bottom rows). Bar, top, 120 μ m, middle and bottom rows, 30 μ m. **c**, Quantitative analysis of spheres. All resulting spheres were exclusively monochromatic, indicating their monoclonal origin and thus are result of cell division and not cell aggregation. Number (mean \pm s.d.) of EGFP positive (EGFP) and DsRed positive (DsR) spheres in individual groups: EGFP only: EGFP 163.5 ± 25.9 , $n = 6$, DsRed 0; 4 + 1: EGFP 169.7 ± 29.8 , $n = 3$, DsRed 44.3 ± 14.2 , $n = 3$; 1 + 4: EGFP 46.0 ± 19.3 , $n = 3$, DsRed 208.7 ± 69.0 , $n = 3$; DsRed: EGFP 0, DsRed 273.0 ± 62.0 , $n = 3$. The table indicates the number of cells seeded.



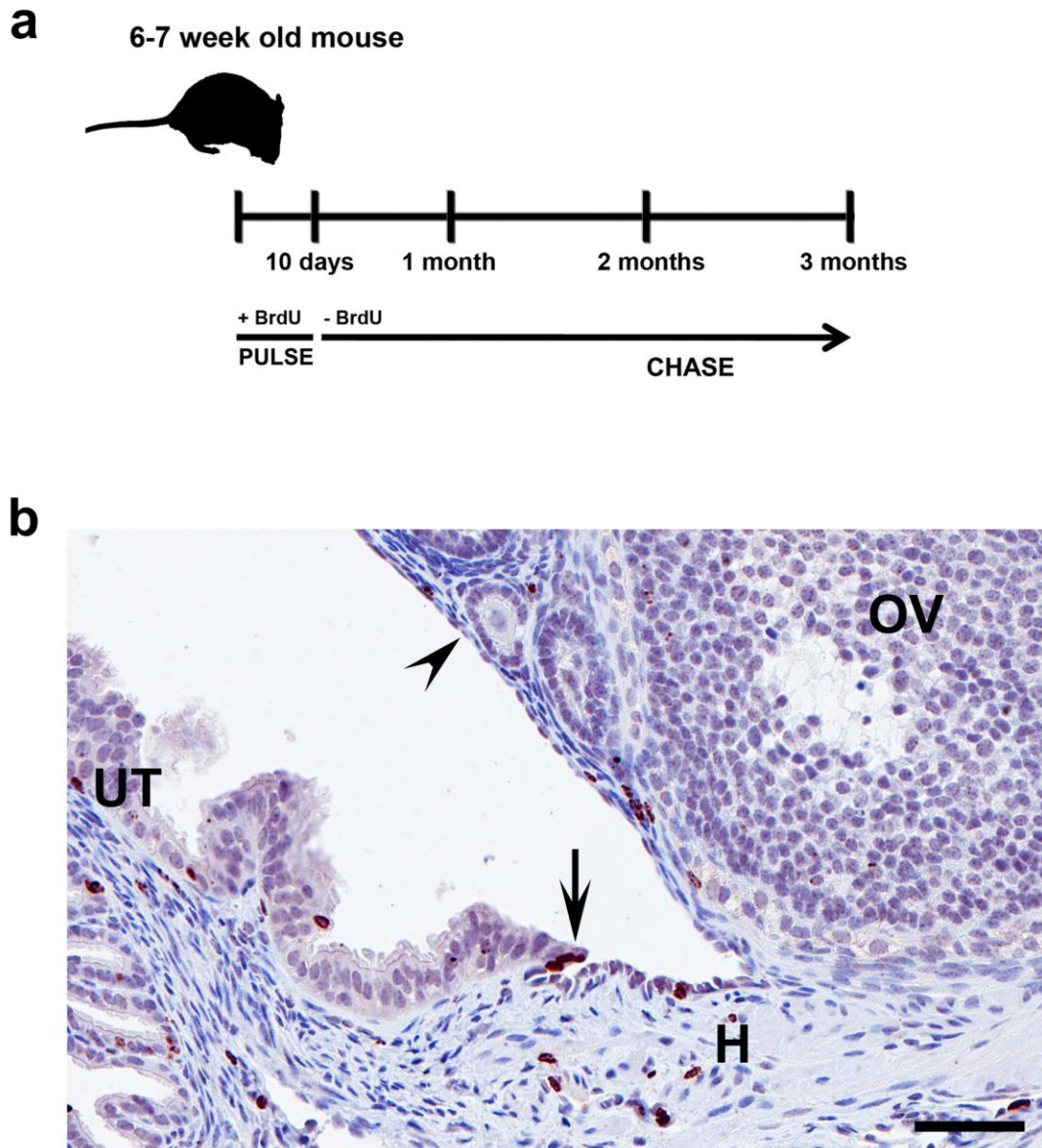
Supplementary Figure 3. Formation of spheres from a single cell. Phase contrast (a, c) and fluorescent (b, d) images of OSE cells isolated from Tg β -actin EGFP mice were imaged immediately and within 43 hrs after placing in Geltrex. Bar, 25 μ m.



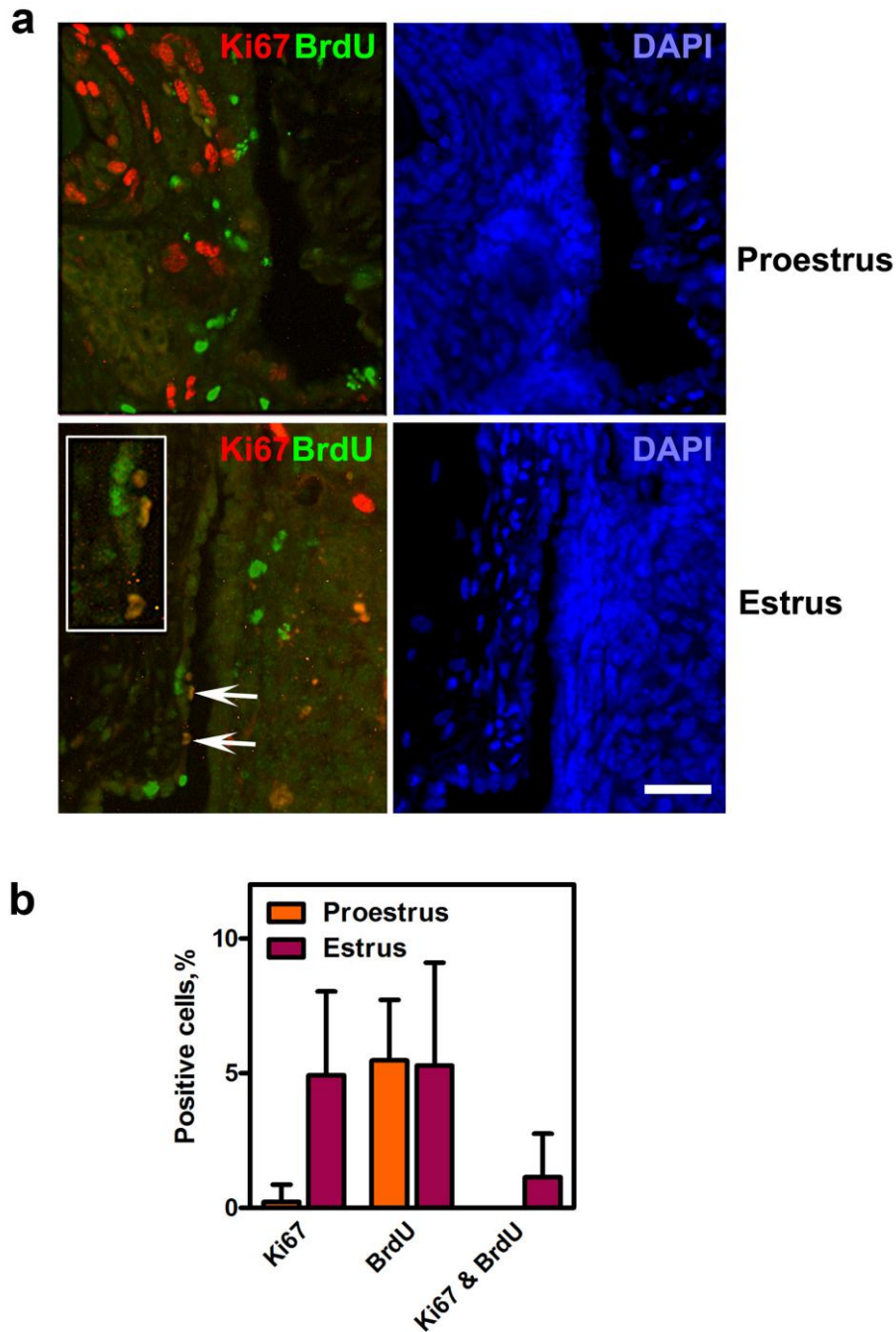
Supplementary Figure 4. Characterization of spheres formed by ALDH⁻ and ALDH⁺ cells grown in Geltrex. **a**, Size of spheres formed by ALDH⁻ versus ALDH⁺ cells ($n=12$, mean \pm s.d., two-tailed $P = 0.0002$) at first generation (G). **b**, Frequency of sphere forming cells and ALDH⁺ cells before placing into culture (G0) and at G1 and G3 ($n = 3$, mean \pm s.d.).



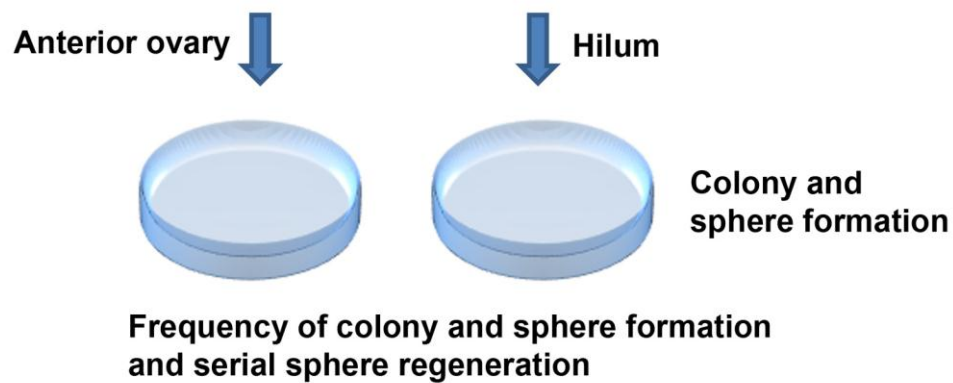
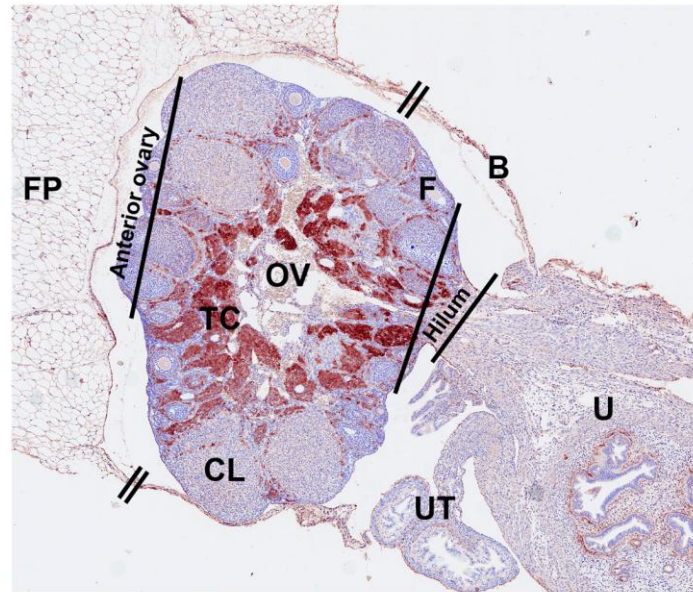
Supplementary Figure 5. Frequency of ALDH1⁺ OSE cells in anatomical regions of the ovary of mice of different genetic backgrounds. Positive and negative cells were counted after ABC-Elite immunohistochemical detection of ALDH1 and hematoxylin counterstaining of 4 μ m thick paraffin sections of ovaries of 60 days old female mice (FVB/N, $n = 6$, other groups, $n = 3$, mean \pm s.d.).



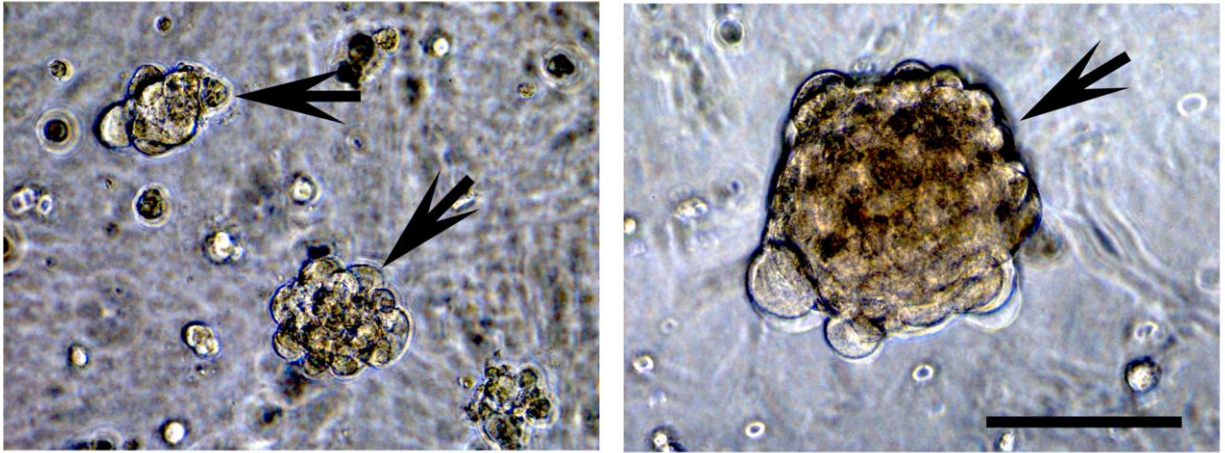
Supplementary Figure 6. BrdU pulse chase experiments. **a**, Schematic of BrdU pulse chase timeline. Animals were injected daily with BrdU for 10 days (pulse) and their ovaries were collected after the pulse and at monthly intervals for 3 months for analysis. **b**, Immunoperoxidase detection of BrdU label retaining cells (brown nuclei) in the hilum area 3 months after BrdU pulse. Arrow, the junction between OSE and tubal epithelium. Arrowhead, OSE; H, hilum; OV, ovary; UT, uterine tube. ABC Elite method. Hematoxylin counterstaining. Bar, 50 μ m.



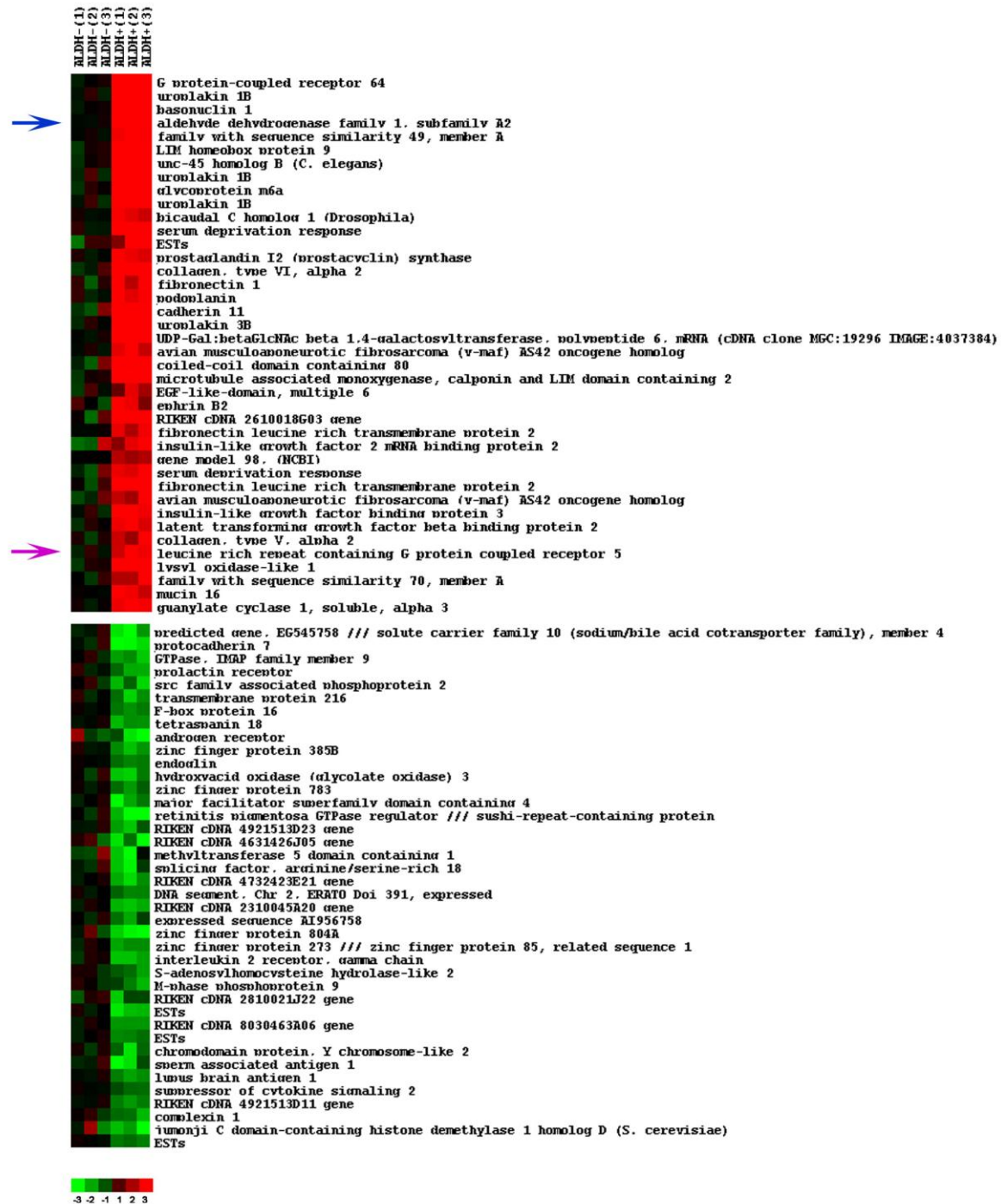
Supplementary Figure 7. Detection of Ki67 and BrdU positive OSE cells in the hilum of mice subjected to BrdU pulse 2 months ago and sacrificed either in proestrus or estrus. a, Yellow fluorescence (arrows and insert in bottom left image) indicates co-localization of Ki67 (red) and BrdU (green). Note that double positive (Ki67 & BrdU) OSE cells are present only in mice collected at estrus but not proestrous stages. Double immunofluorescence. DAPI counterstaining, blue. Bar, all large images, 30 μ m, insert, 13 μ m. **b,** Quantification of hilum cells positive for Ki67, BrdU or both ($n = 8$, mean \pm s.d.). Proestrus versus Estrus two tailed P : Ki67, 0.0008; BrdU, 0.9077; Ki67 & BrdU, NA, Proestrus column is zero.



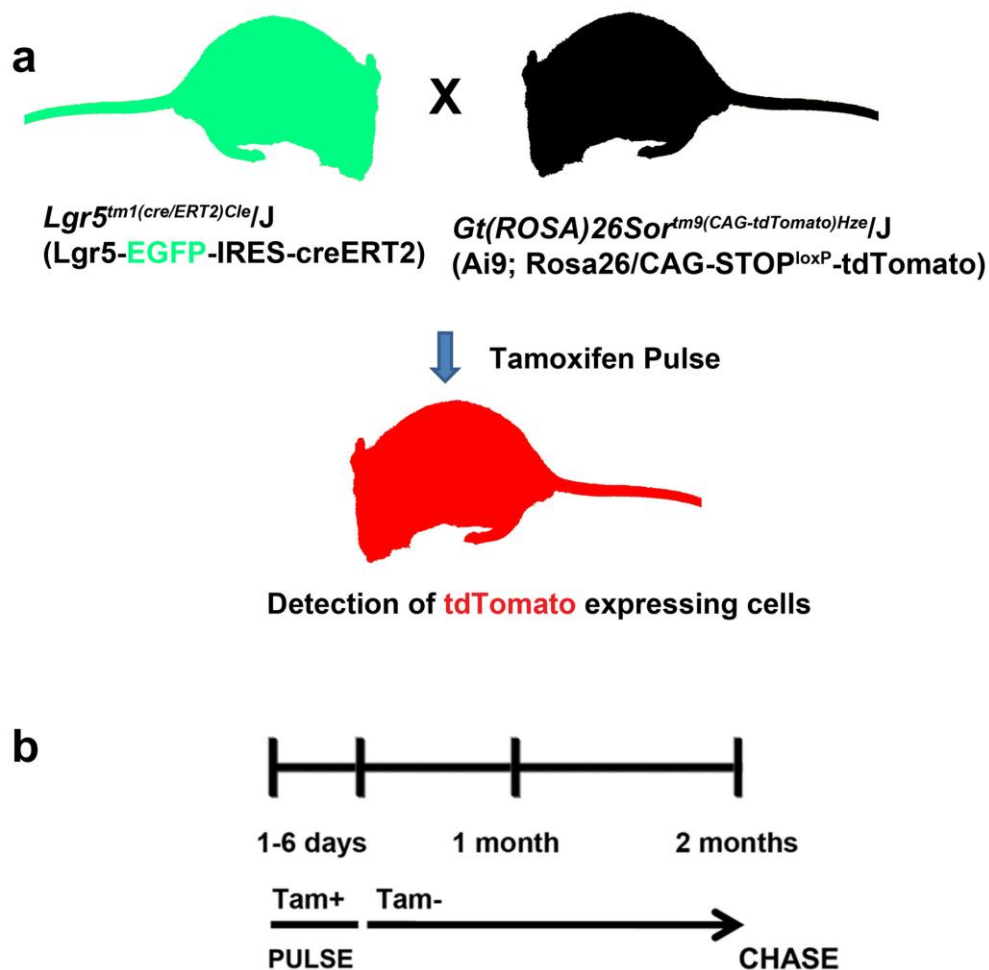
Supplementary Figure 8. Schematic of isolation of OSE cells from the hilum and the ovary part (anterior ovary) under dissection microscope. B, bursa; CL, corpus luteum; F, follicle; FP, fat pad, OV, ovary; TC, theca cells; UT, uterine tube; U, uterus.



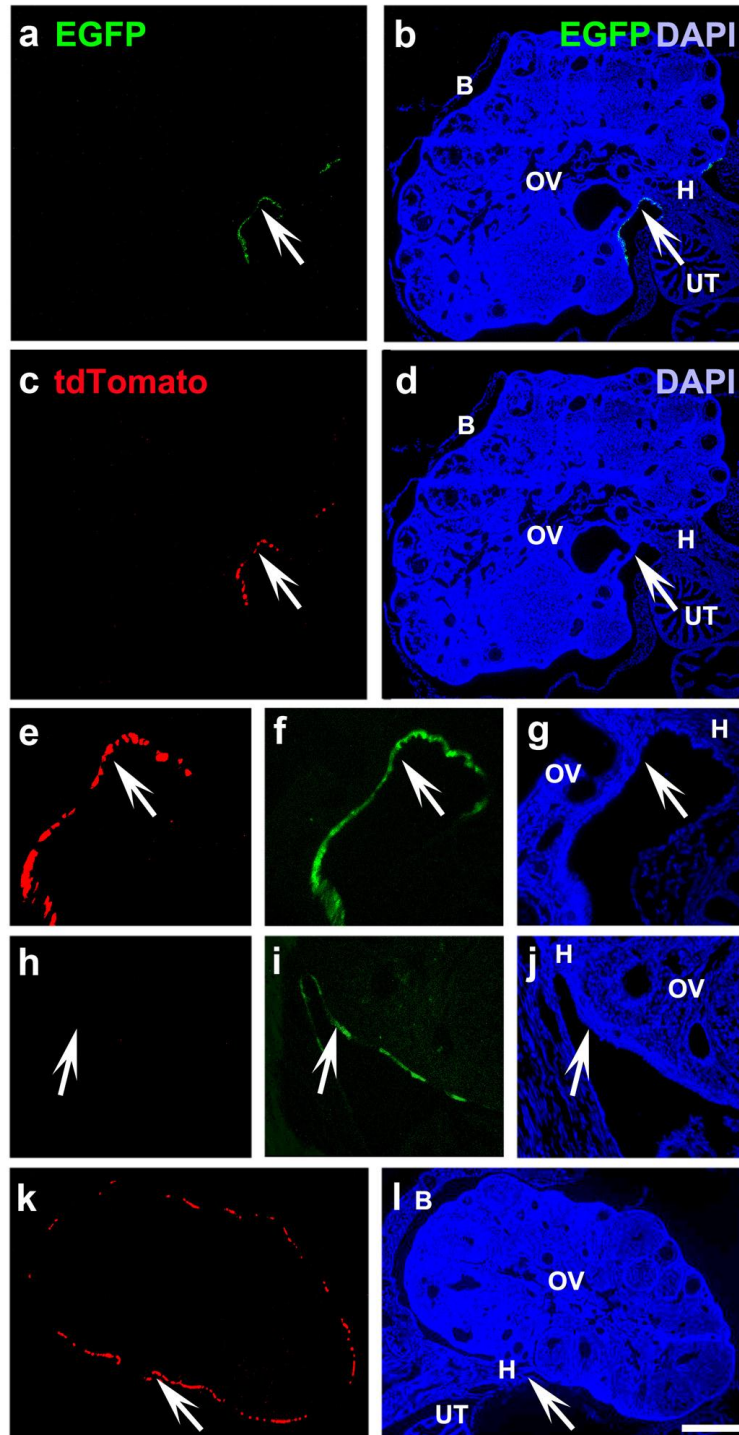
Supplementary Figure 9. Images of OSE spheres (arrows) formed after microdissection of the hilum region. Phase contrast. Bar, both images, 130 μm .



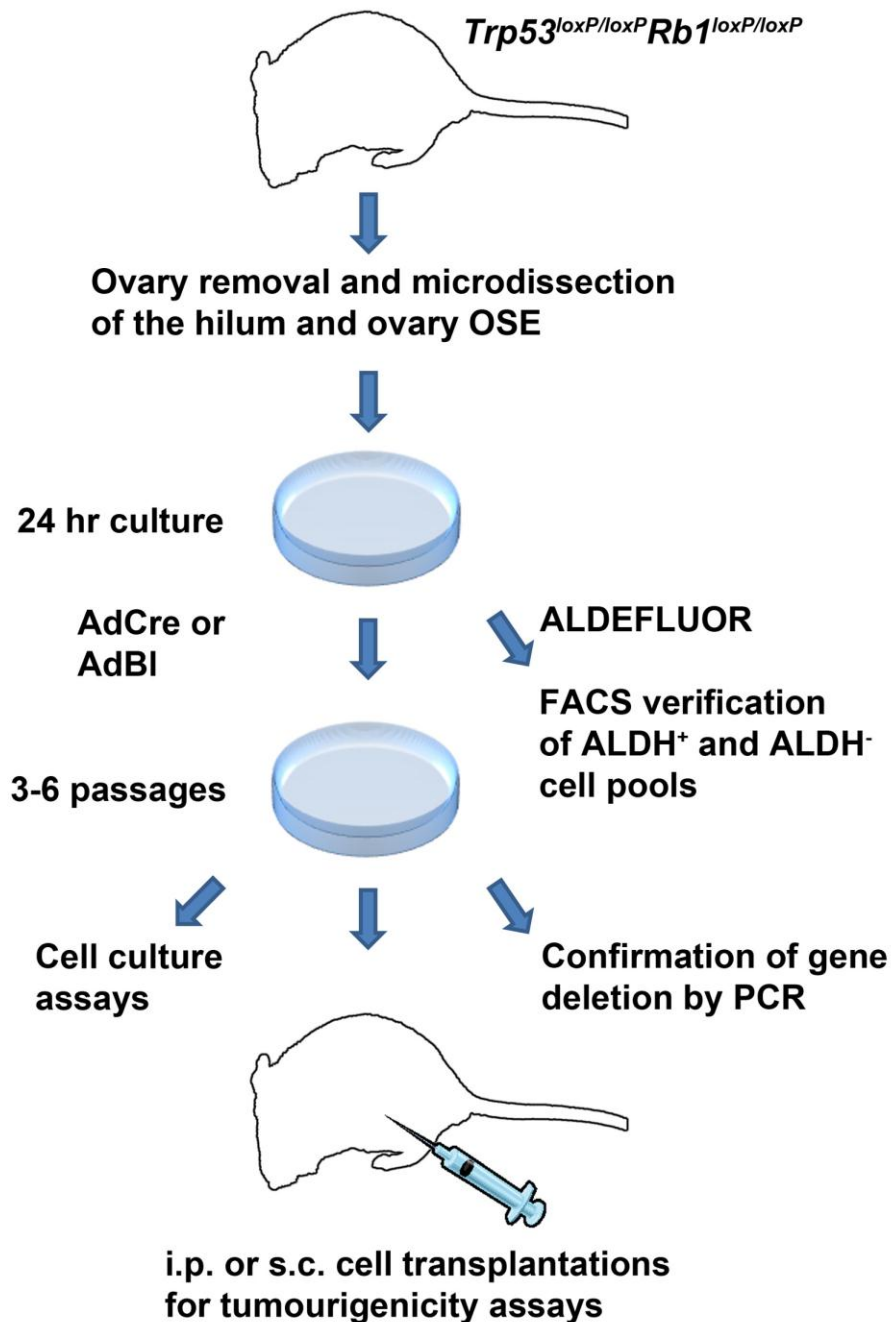
Supplementary Figure 10. Expression profiles of top 80 genes differentially expressed between ALDH⁺ and ALDH⁻ cells. Expression of *Aldh1* and *Lgr5* genes are indicated by blue and magenta arrows, respectively. Three independent pools (10 mice each) of ALDH⁺ and ALDH⁻ cells were analyzed. Significantly upregulated and downregulated genes were sorted by the order of significance at t-test level of 0.01 and 0.05, respectively. Affymetrix Mouse Genome 430 2.0 array.



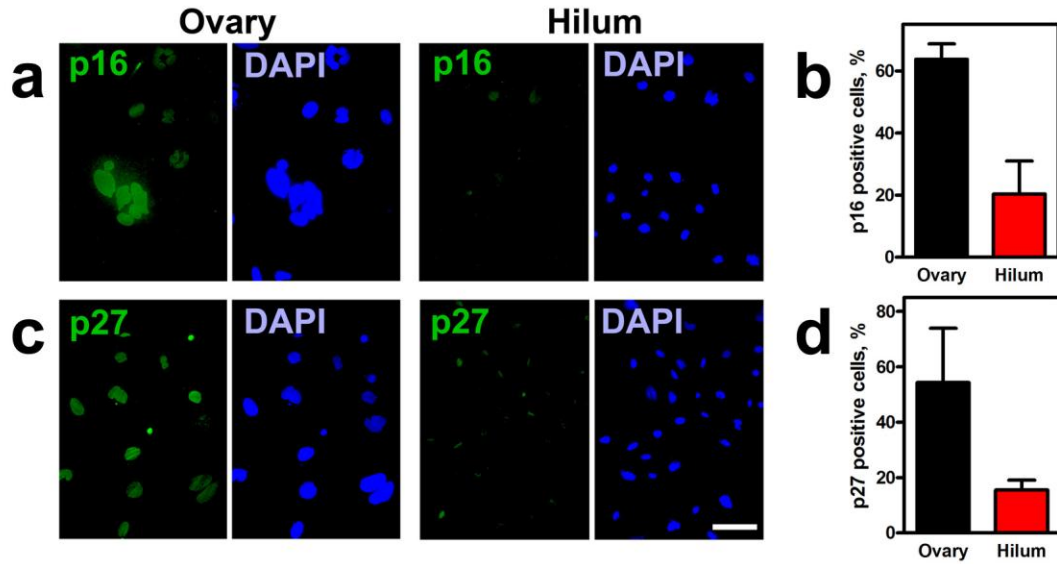
Supplementary Figure 11. Design of lineage tracing experiment. **a**, *Lgr5^{tm1(cre/ERT2)Cle/J}* (Lgr5-EGFP-IRES-creERT2) male mice, which harbor a Lgr5-EGFP-IRES-CreERT2 "knock-in" allele, were bred with *Gt(ROSA)26Sor^{tm9(CAG-tdTomato)Hze/J}* (Ai9) homozygous female mice, and the resulting female offspring, which carried both Lgr5 and Ai9 genetic modifications, were used in lineage tracing experiments. Note that while EGFP expression (green) under *Lgr5* promoter is constitutive, expression of *Rosa26/CAG* promoter-driven red fluorescent protein variant (tdTomato, red) is possible only after Cre-mediated deletion of the *STOP* codon flanked by *loxP* sites. **b**, Timeline of Tamoxifen (Tam) pulse chase experiment. Mice were injected intraperitoneally with a single dose of Tam or three times every second day for 6 days. Ovaries of Tam-pulsed mice were collected 1 and 3 days after the pulse and at monthly intervals for 2 months.



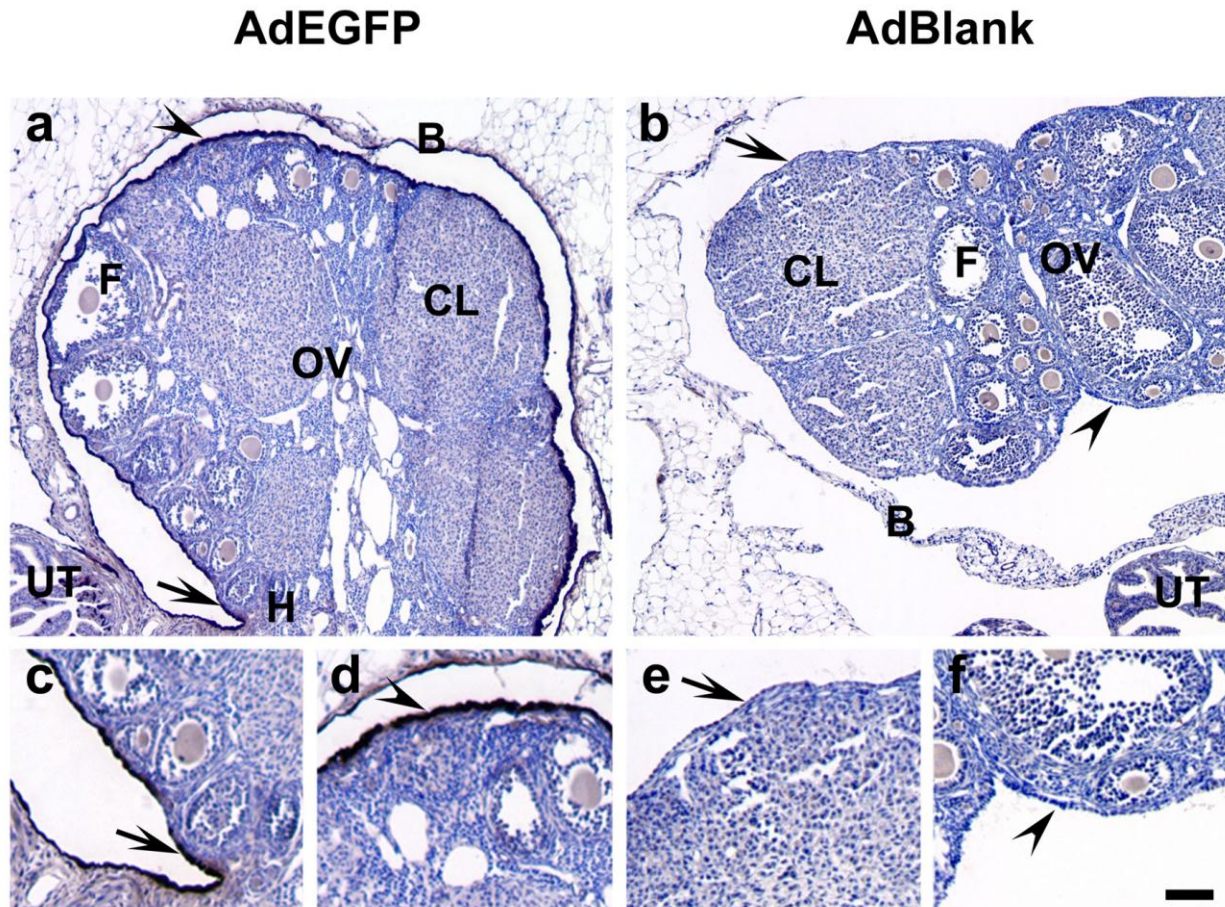
Supplementary Figure 12. Tracing the fate of the $Lgr5^{+}$ hilum cells. a-l. Detection of EGFP (green, a, b, f, i), tdTomato (red, c, e, h, k) expression and DAPI (blue, b, d, g, j, l) in the ovaries of $Lgr5$ -EGFP-IRES-creERT2 Ai9 mice 1 day (a-j) and 1 month (k-l) after administration of either tamoxifen (a-g, k, l) or vehicle (h-j). Arrows, hilum OSE; B, bursa; H, hilum; OV, ovary; UT, uterine tube. Bar, a-d, k, l, 600 μ m, e-j, 130 μ m.



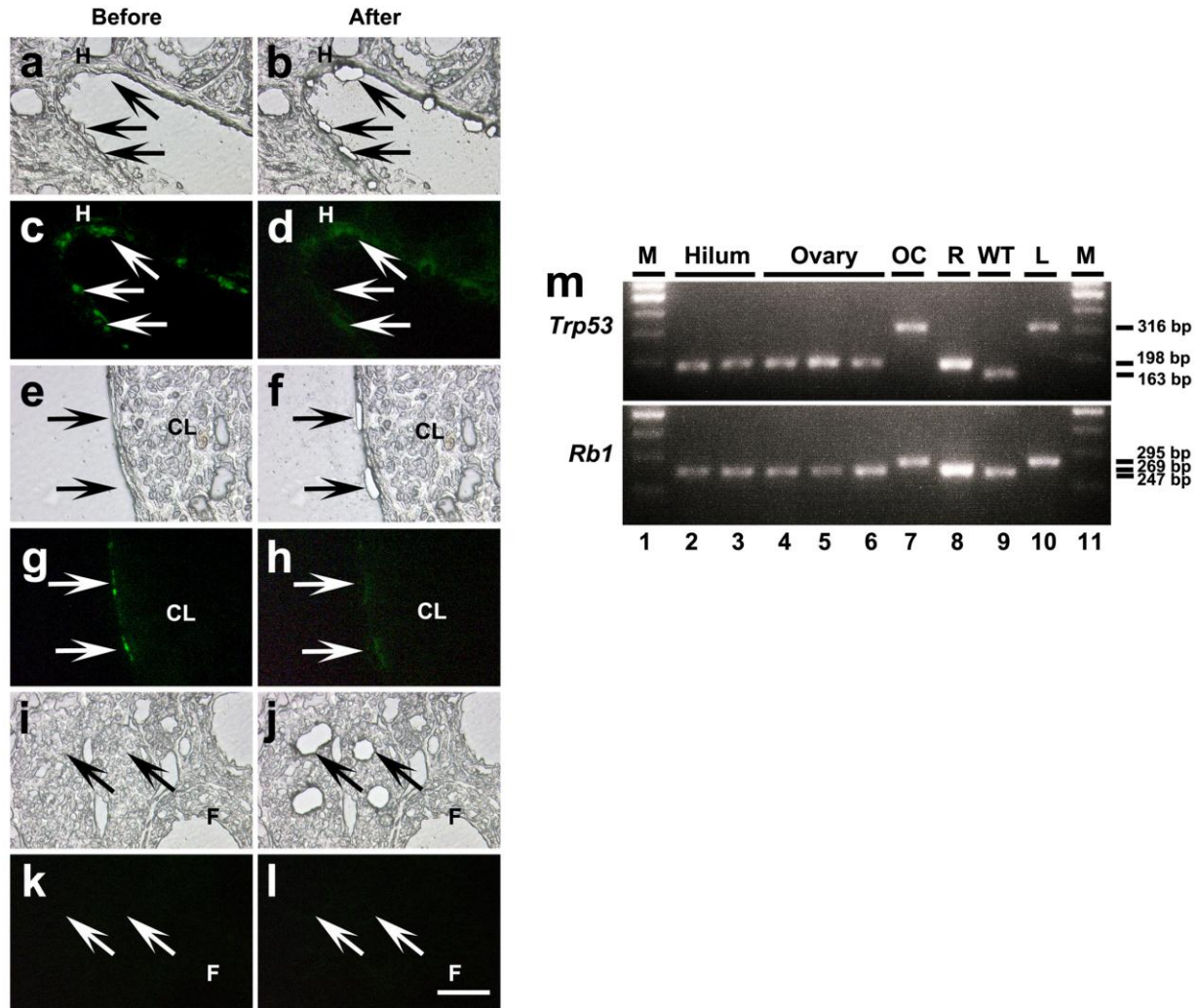
Supplementary Figure 13. Design of experiments for evaluation of effects of Cre-LoxP mediated inactivation of *Trp53* and *Rb1* on OSE cell subpopulations. The ovaries were removed from *Trp53^{loxP/loxP}Rb1^{loxP/loxP}* mice, and cells microdissected from the hilum and remainder of the ovary (ovary) were further purified by differential adhesion in 24 hr cell culture, followed by FACS analysis of cell pool composition. Cultures containing at least 98% ALDH1⁺ cells and 97% ALDH1⁻ cells isolated from the hilum and ovary regions, respectively, were exposed to either AdCre or AdBlank (AdBI), and used for cell culture and in vivo tumourigenicity assays. Deletion of *Trp53* and *Rb1* was confirmed by PCR genotyping.



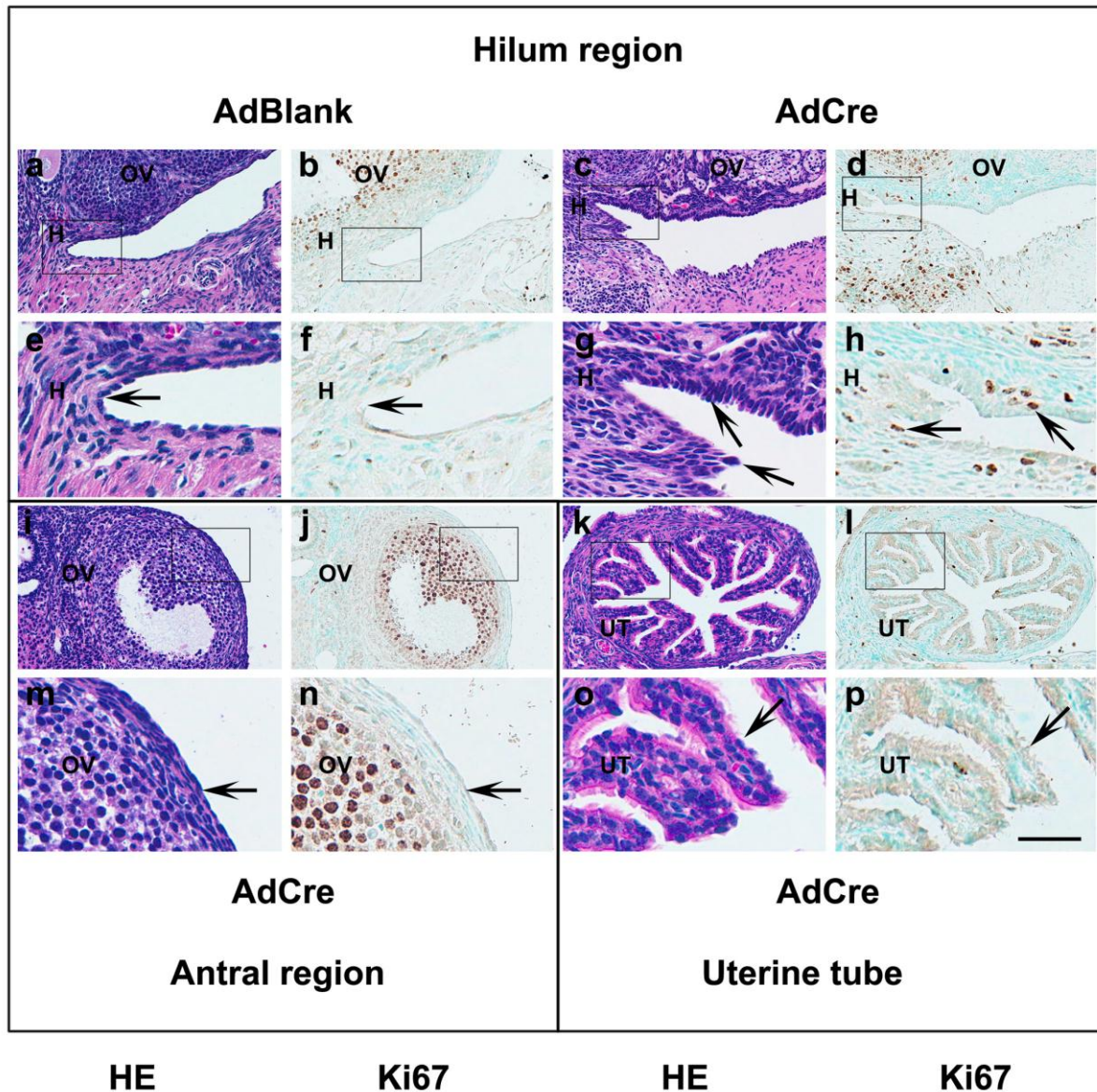
Supplementary Figure 14. Expression of senescence markers in primary OSE cells isolated from the hilum and the remainder of the ovary (Ovary) and evaluated at passage 6 after *Cre-loxP* mediated inactivation of *Trp53* and *Rb1*. Detection (a, c) and quantitative analysis (b, d) of p16 (a, b) and p27 (c, d) expression in the ovary and hilum cells (b, $n = 3$, mean \pm s.d, $P = 0.003$; d, $n = 3$, mean \pm s.d, $P = 0.0283$). Counterstaining with DAPI, blue. Bar, 110 μ m.



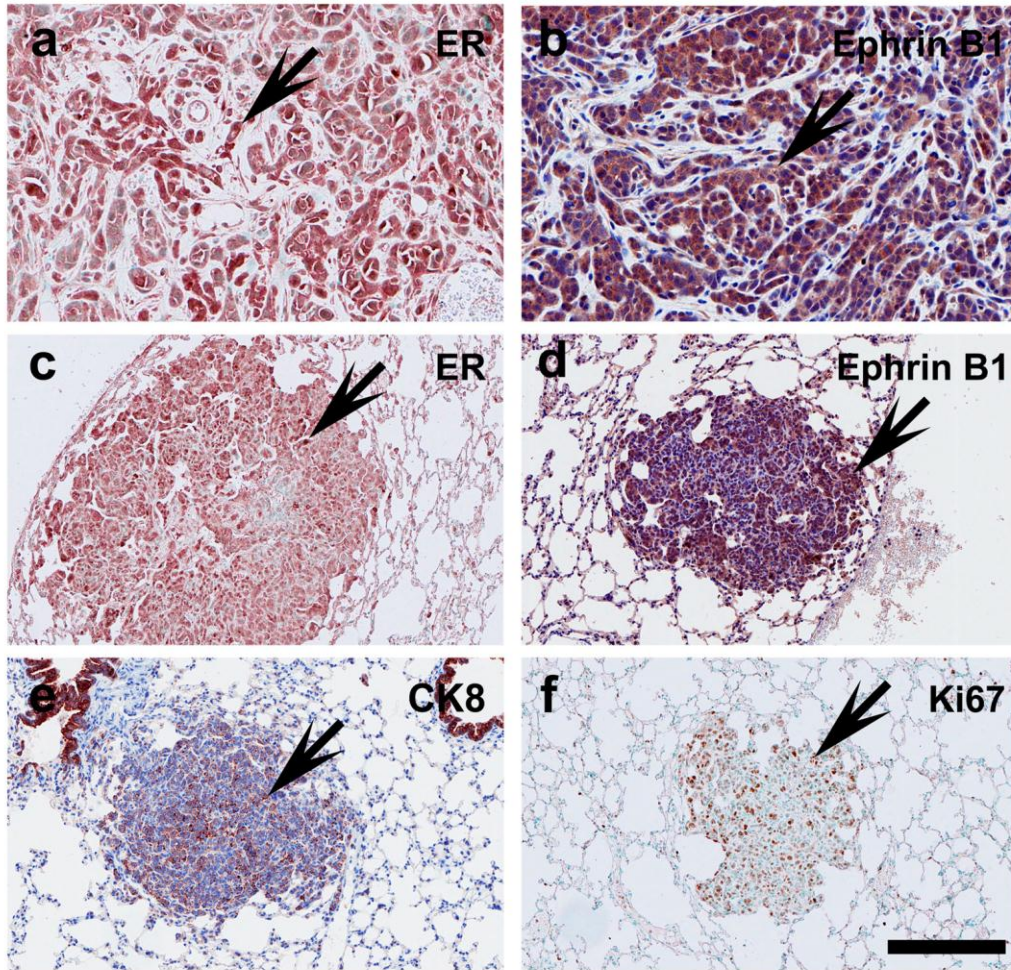
Supplementary Figure 15. EGFP expression in the OSE exposed to AdEGFP or AdBlank delivered by a single trans-infundibular intrabursal injection. a-f, Uniform staining (brown color) of the OSE in all areas after exposure to AdEGFP (a, c, d) but not AdBlank (b, e, f). Arrows and arrowhead indicate respective regions in low (a, b) and high (c-f) magnification images. B, bursa; CL, corpus luteum; F, follicle; H, hilum; OV, ovary; UT, uterine tube. ABC Elite method. Hematoxylin counterstaining. Bar, a, b, 100 μ m, c-f, 50 μ m.



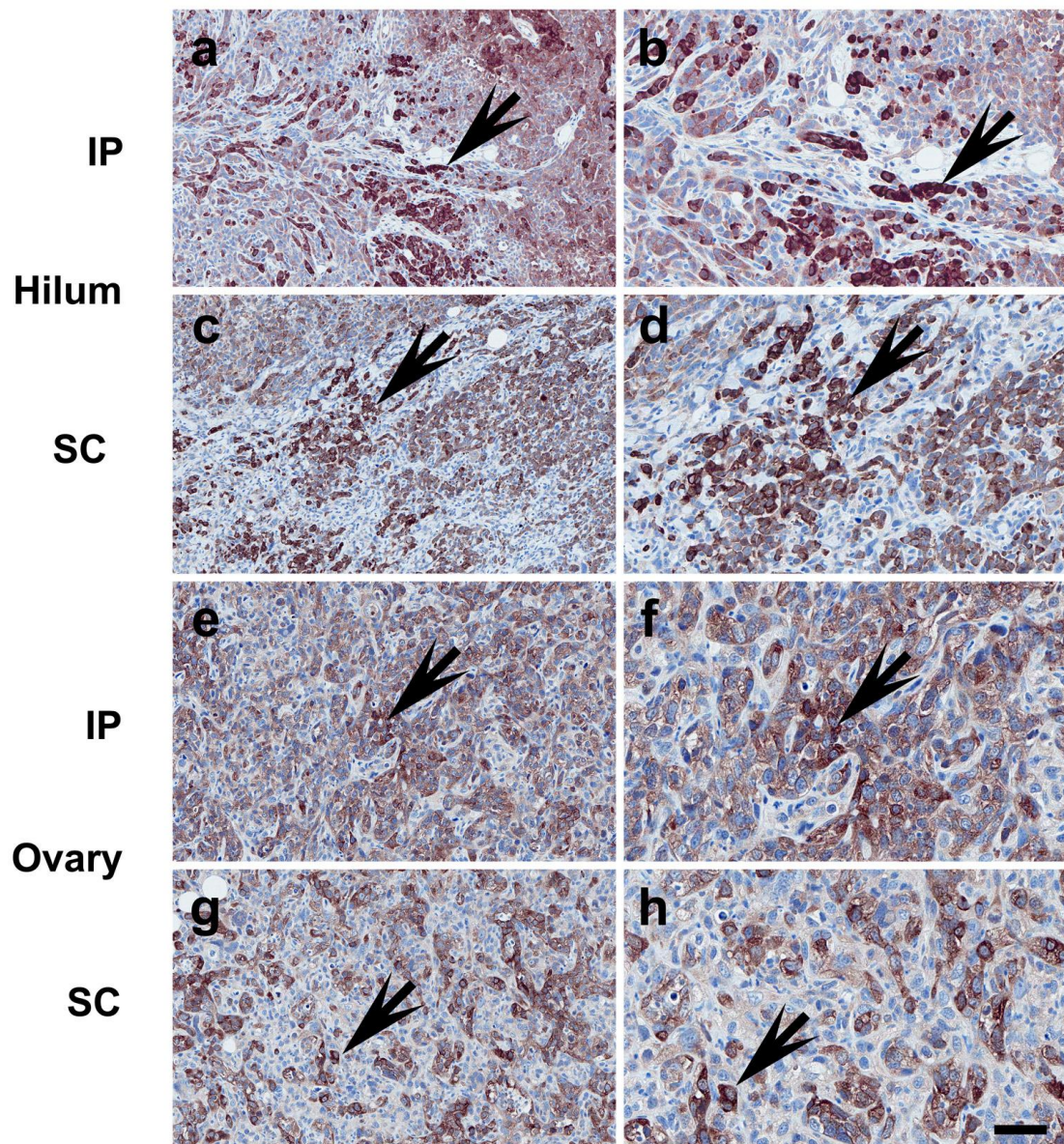
Supplementary Figure 16. Detection of *Trp53* and *Rb1* deletion in various regions of the OSE 2 days after trans-infundibular administration of AdCre-EGFP. a - l, OSE of the hilum (a-d), and CL (e-h) regions, and ovary core (i-l) before (a, c, e, g, i, k) and after (b, d, f, h, j, l) microdissection of regions either expressing (green, hilum and CL regions) or not expressing EGFP. Phase contrast (a, b, e, f, i, j) and fluorescence (c, d, g, h, k, l). CL, corpus luteum; F, follicle; H, hilum. Bar, a-l, 100 μ m. **m, PCR analysis of *Trp53* and *Rb1* gene structure in the same samples laser microdissected from the OSE of the hilum (Hilum, lanes 2, 3), and the rest of ovary (Ovary, lanes 4-6), and from the ovarian core (OC, lane 7). Samples from with known gene structure (homozygous recombinant gene, R, lane 8; wild-type, WT, lane 9; and floxed gene, L, lane 10). 316-, 198-, and 163-bp fragments are diagnostic for floxed, excised, and wild-type alleles of the *Trp53* gene, respectively. 295-, 269-, and 247-bp fragments are diagnostic for floxed, excised, and wild-type alleles of the *Rb1* gene, respectively. M (Lane 1 and 11), DNA marker.**



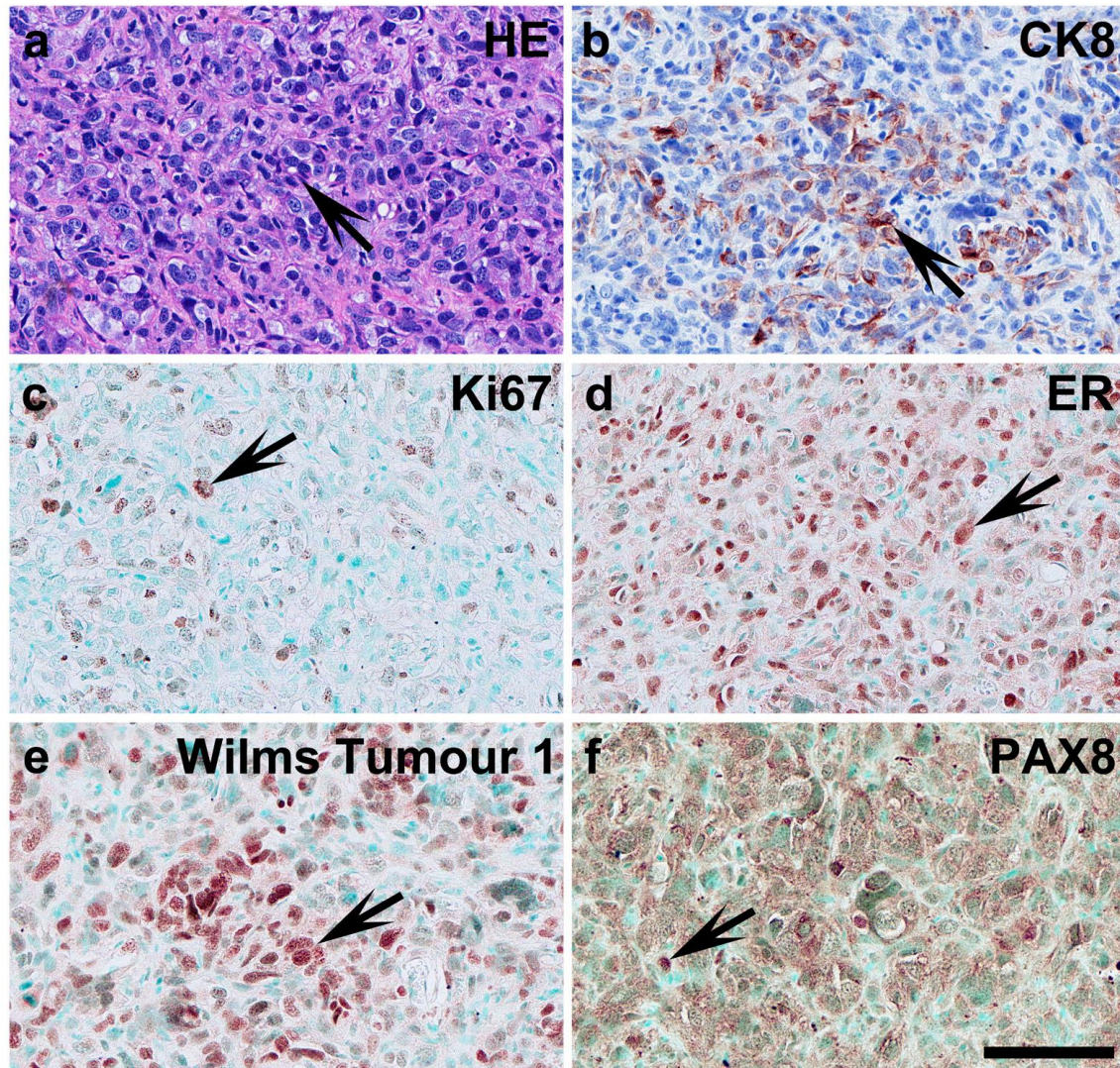
Supplementary Figure 17. Epithelia in the hilum and antral regions of the ovary and the uterine tube. a-p, Low (a-d, i-l) and high (e-h, m-p) magnification images of indicated by rectangle regions of *Trp53^{loxP/loxP}Rb1^{loxP/loxP}* mouse ovary 60 days after trans-infundibular intrabursal injection of AdCre or AdBlank. Early atypical lesions in the hilum region (c, d, g, h) are characterized by increased nuclear to cytoplasmic ratio, nuclear size and shape variability, more columnar appearance, cell crowding (c, g, arrows) and increased number of Ki67 positive cells (brown nuclei, d, h). No atypical cells are detected in the epithelium (arrows) of the hilum region after exposure to AdBlank (a, b, e, f) and epithelia (arrows) of the antral ovarian region (i, j, m, n) and the uterine tube (k, l, o, p) after exposure to AdCre. Rectangles (a-d, i-l) indicate areas shown in the higher magnification images (e-h, m-p). HE, Hematoxylin and eosin; Ki67, ABC Elite method, methyl green counterstaining. H, hilum, OV, ovary, UT, uterine tube. Bar, a-d, i-l, 100 μ m, e-h, m-p, 30 μ m.



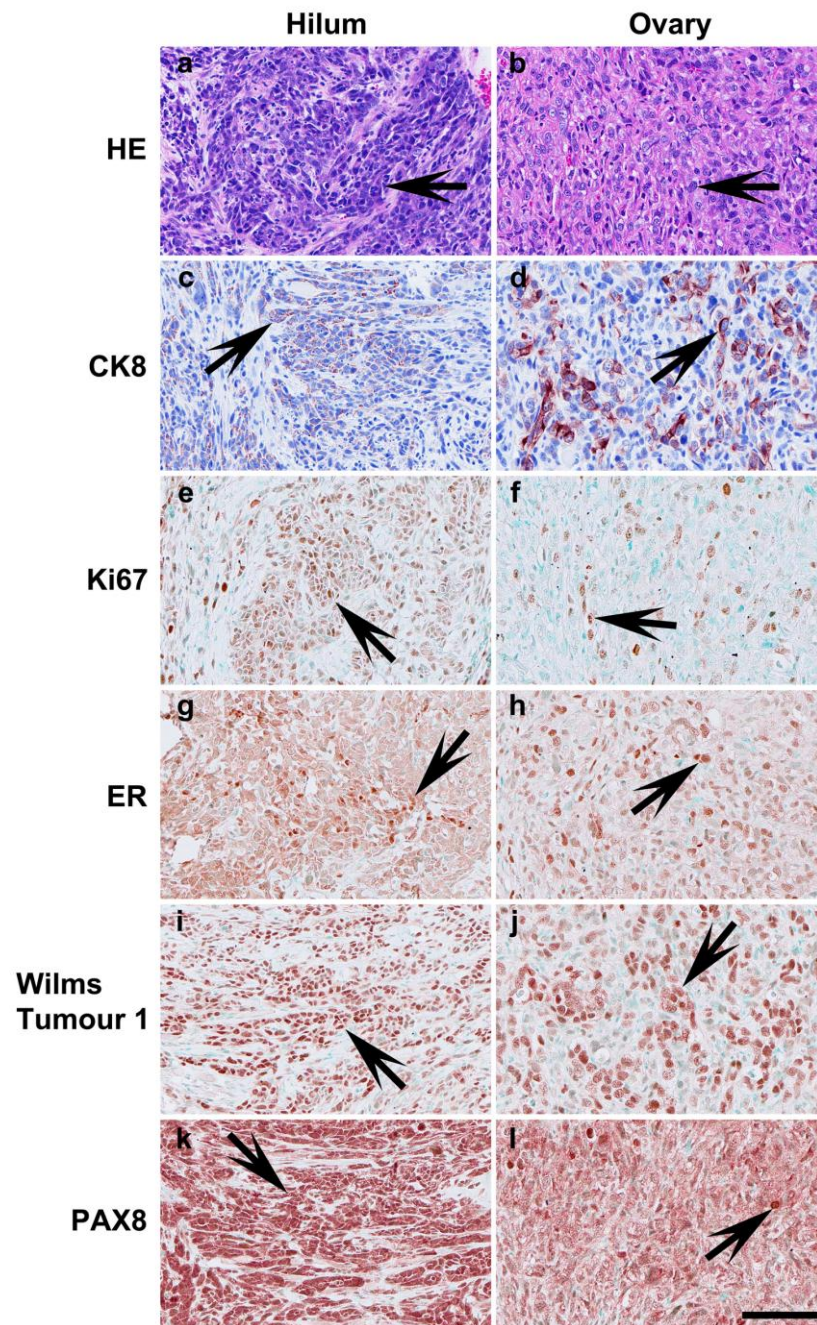
Supplementary Figure 18. Characterization of neoplasms arising from *Trp53* and *Rb1* deficient hilum cells transplanted into the mouse abdominal cavity. a-f, Expression (arrows) of Estrogen Receptor α (ER, a, c), Ephrin B1, (b, d), cytokeratin 8 (CK8) and Ki67 in neoplastic cells of primary tumour (a-b) and lung metastasis (c-f). ABC Elite method. Counterstaining with hematoxylin (a-e) and methyl green (f). Bar, a-c, 100 μ m, d-f, 200 μ m.



Supplementary Figure 19. Expression of ALDH1 in neoplasms arising from *Trp53* and *Rb1* deficient OSE cells. a-f, OSE cells derived from the hilum (Hilum, a-d) or remainder of the ovary (Ovary, e-h) were transplanted intraperitoneally (IP, a, b, e, f) or subcutaneously (SC, c, d, g, h). Low (a, c, e, g) and high (b, d, f, h) magnification images of regions containing neoplastic cells with high ALDH1 expression (dark brown, arrows). Neoplasms derived from hilum cells contained more ALDH1 highly positive cells ($n = 6$; mean \pm s.d., $22.5 \pm 5.2\%$), as compared to those formed from the remainder OSE cells ($n = 2$, mean \pm s.d., $11 \pm 7.1\%$), $P = 0.0437$. ABC Elite method. Counterstaining with hematoxylin. Bar, a, c, e, g, 100 μ m, b, d, f, h, 50 μ m.



Supplementary Figure 20. Characterization of neoplasm arising from *Trp53* and *Rb1* deficient non-hilum ovary (remainder of the ovary) OSE cells transplanted into the mouse abdominal cavity. a Carcinoma with mainly solid pattern of growth and occasional gland-like structures (arrow), **b-f**, Expression (arrows) of cytokeratin 8 (CK8, b), Ki67 (c), Estrogen Receptor α (ER, d), Wilms Tumour 1 (e), and PAX8 (f) in neoplastic cells. Note that only few cells have nuclear PAX8 expression (arrow). Hematoxylin and eosin (HE, a), ABC Elite method. Counterstaining with hematoxylin (b) and methyl green (c-f). Bar, a-f, 100 μ m.



Supplementary Figure 21. Characterization of neoplasm arising from *Trp53* and *Rb1* deficient hilum and ovary (non-hilum, remainder of the ovary) OSE cells transplanted subcutaneously. **a, b** Neoplastic cells form nested and gland-like (a, arrow) or solid (b) patterns. **c-l**, Expression (arrows) of cytokeratin 8 (CK8, c, d), Ki67 (e,f), Estrogen Receptor α (ER, g, h), Wilms Tumour 1 (i, j), and PAX8 (k, l), in neoplastic cells. Note that only few cells have nuclear PAX8 expression in neoplasm from ovary OSE cells (l, arrow). Hematoxylin and eosin (HE, a, b), ABC Elite method. Counterstaining with hematoxylin (c, d) and methyl green (e-l). Bar, a-l, 100 μ m.

Supplementary Tables

Supplementary Table 1. Frequency of sphere forming cells (SFC) in ALDH⁺ and ALDH⁻ OSE cell subpopulations at generation 1*

Cell subpopulation	ALDH ⁺	ALDH ⁻
Population size, % \pm s.d.	5.27 \pm 0.55	94.73 \pm 0.55
Number of SFC per 10 ⁴ cells of ALDH ^{+/+} subpopulations \pm s.d.	143 \pm 30	0.2 \pm 0.04
SFC frequency% \pm s.d.	1.43 \pm 0.30	0.002 \pm 0.0004
Absolute SFC number per 10 ⁴ cells of total OSE population, \pm s.d. [#]	7.53 \pm 0.16	0.19 \pm 0.0002
Distribution of SFC, %	97.54	2.46

*All experiments were independently repeated 5 times. Wilcoxon Exact Test P = 0.0083 in all ALDH⁺ vs. ALDH⁻ groups.

[#]Absolute number of SFC is calculated as [number of spheres formed by cell subpopulation/input cell number] x number of cells in subpopulation per 10⁴ cells in total population.

Supplementary Table 2. Frequency of sphere forming cells (SFC) isolated by microdissection from the ovarian hilum and propagated for 7 consecutive generations (G, dissociation/clonal formation) in a representative experiment

Generation	1	2	3	4	6	7
Input cell number	1×10^5	1×10^5	1×10^5	1×10^5	0.59×10^5	1.18×10^5
Replicates number	6	5	6	4	2	3
SFC, absolute number	8.4	14.2	25.8	21.3	27.5	29.0
SFC, %	0.008	0.014	0.026	0.021	0.047	0.025

Culturing of cells isolated from the anterior region of the ovary resulted in 0.12 spheres per 1×10^5 ($n = 6$), that is 0.00012%. These spheres very rarely formed spheres in G2 and did not yield any spheres in G3.

Supplementary Table 3. List of genes tested for expression in ALDH⁺ and ALDH⁻ OSE cells by qRT-PCR

Gene name	Sequence (5' → 3')	
	Forward Primer	Reverse Primer
Bmi1 F	TGGTTGTTTCGATGCATTTCT	CTTTCATTGTCTTTTCCGCC
Catnb1	CAGCTTGAGTAGCCATTGTCC	GAGCCGTCAGTGCAGGAG
Cd133	TAGAGGGAAGTCATTTCGGCT	CCCAAGATACCTTCAATGCTG
Cd44	AGCGGCAGGTTACATTCAAA	CAAGTTTTGGTGGCACACAG
Dll1	CTCCCCTGGTTTGTACAGT	GGAGAAGATGTGCGACCCT
Gapdh	TTGATGGCAACAATCTCCAC	CGTCCCGTAGACAAAATGGT
Jag1	GGCGAAACTGAAAGGCAGTA	GCTTCGGCTCAGGGTCTAC
Klf4	CAGTGGTAAGGTTTCTCGCC	GCCACCCACACTTGTGACTA
Krt6a	CCTGAAGAAGGATGTAGATGCTG	GAGACAGTTCTGCTTCATAGAGAG
Krt6b	CGTGAAAAAGGATGTAGATGCTGC	GAGACAGTTCTGCCTCATAAATAAC
Lef1	CTCGTCGCTGTAGGTGATGA	AAATGGGTCCCTTTCTCCAC
Lgr5	TCTTCTAGGAAGCAGAGGCG	CAACCTCAGCGTCTTCACCT
Lgr6	AGGGAAGTTGGCCCTGTCTC	GGATGAAAGTCCTCGGCCTG
Lmyc	TCCAGAGATCGCCTCTTCTC	AGACTCAGGCCTGCTCCG
Lrp6	ATCGTTGCATTCTCTTTGCC	TCTGCGTGCTGCTGAGAG
Met	TGTCCGATACTCGTCACTGC	CATTTTACGGACCCAACCA
Myc	TGAAGTTCACGTTGAGGGG	AGAGCTCCTCGAGCTGTTTG
Nanog	AGGGTCTGCTACTGAGATGCTCTG	CAACCACTGGTTTTTCTGCCACCG
Nmyc1	TCTCTACGGTGACCACATCG	AGCACCTCCGGAGAGGATA
Notch1	CTGAGGCAAGGATTGGAGTC	GAATGGAGGTAGGTGCGAAG
Notch2	TGTGCCGTTGTGGTAGGTAA	TGCTGTGGCTCTGGCTGT
Snai1	AGTGGGAGCAGGAGAATGG	CTTGTGTCTGCACGACCTGT
Sox2	AAAGCGTTAATTTGGATGGG	ACAAGAGAATTGGGAGGGGT
Wnt1	GAAGATGAACGCTGTTTCTCG	AAATGGCAATTCCGAAACC
Wnt3	GAGGCCAGAGATGTGTACTGC	CTTCTAATGGAGCCCCACCT
Wnt5a	CCGGGCTTAATATTCCAATG	ACGCTTCGCTTGAATTCCT
Wnt8a	GGATGGCATGAATGAAGGAT	GGTGGAATTGTCCTGAGCAT

Supplementary Table 4. Frequency and distribution of early atypical lesions in mouse OSE and tubular epithelium 60 days after *Cre-LoxP* mediated inactivation of *Trp53* and *Rb1*

Total number of mice	Number of mice with lesions	Number of lesions per region				
		Antral	CL	Distal	Hilum	Uterine tube
16	12	0	0	0	13	0

All early atypical lesions were diagnosed based on their morphology, staining for Ki67 and detection of floxed out *Trp53* and *Rb1* as described earlier ¹. In one case, two independent hilum lesions were detected, as confirmed by serial sections followed by 3D reconstruction.

Supplementary Table 5. Frequency of tumour formation and lung metastasis after subcutaneous transplantation of serially diluted *Trp53* and *Rb1* deficient ovary (non-hilum) and hilum OSE cells

Number of transplanted cells	Number of subcutaneous tumours/total number of transplantations (%)		Number of mice with lung metastasis/ number of tumour bearing mice (%)	
	Ovary	Hilum	Ovary	Hilum
5×10^4	0/4 (0%)	4/4* (100%)	NA [#]	4/4(100%)
5×10^5	0/4 (0%)	3/4 (75%)	NA	3/3(100%)
5×10^6	1/6 (17%)	4/4* (100%)	0/1 (0%)	2/4 (50%)

*Fisher's Exact Test $P < 0.05$, [#]NA, not applicable. All tumors were collected after reaching 1 cm in diameter.

Supplementary Table 6. Primary antibodies used for immunohistochemistry

Antigen	Protocol	Dilution of antibodies	Antibody source; catalogue number	Antigen retrieval
ALDH1	P	1:2000	Abcam, Cambridge, MA; ab23375	
BrdU	PFC	1:50	BD Biosciences; San Jose, CA, 347580	
BrdU	P	1:100	Abcam; ab2284	CB
CD133	P	1:50	Miltenyi Biotec, Auburn, CA; 130-090-422	CB
CK6b	P	1:100	Proteintech Group, Chicago, IL; 17391-1-AP	CB
CK8	P	1:10	Developmental Studies Hybridoma Bank, Iowa City, IA; TROMA-I	CB
EGFP	P	1:500	Novus Biologicals, Littleton, CO	CB
Ephrin B1	P	1:100	Santa Cruz, Santa Cruz, CA; sc-1011	CB
ER α	P	1:1000	Santa Cruz; sc-542	CB
Ki67	P	1:200	Leica Microsystems; Bannockburn, IL; NCL-Ki67p	CB
Lef1	F	1:50	Cell Signaling, Danvers, MA; 2230S	
P16	PFC	1:100	Santa Cruz; sc-1207	
P27	PFC	1:100	Santa Cruz; sc-528	
PAX8	P	1:100	Proteintech Group 10336-1-AP	CB
Wilms Tumour 1	P	1:200	Santa Cruz; sc-192	CB

Antibodies are used with frozen (F) or paraffin (P) sections of 4% paraformaldehyde fixed tissues or with paraformaldehyde fixed cells (PFC) with or without Citric Buffer (CB) antigen retrieval.